Docket Number EPA-HQ-OPP-2015-0266
www.regulations.gov

MTI Interim Registration Review Decision

Case Number 5018

March 2016

Approved by: _____

Steve Knizner
Director
Antimicrobials Division

Date: 2/26/16

MTI Registration Review Team

Human Health & Environmental Effects
Pat Jennings
Jim Breithaupt
Bill Erickson
Jonathan Leshin
Timothy Dole
Sumitra Biswas

Risk Management
Rachel Ricciardi
Rose Kyprianou

Office of General Counsel
Philip Ross

TERMS, ABBREVIATIONS AND SYMBOLS

A.I. or a.i.	active ingredient
ASRI	activated sludge respiration inhibition
CAS	Chemical Abstracts Service
CFR	Code of Federal Regulations
COC	concentration-of-concern
EC_{50}	median (or 50 percent) effect concentration
EDSP	Endocrine Disruptor Screening Program
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
FFDCA	Federal Food, Drug, and Cosmetic Act
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FQPA	Food Quality Protection Act
Kow	octanol-water partition coefficient
LC_{50}	median (or 50 percent) lethal concentration
LD_{50}	median (or 50 percent) lethal dose
Log K_{ow}	logarithm of the octanol-water partition coefficient
µg	microgram
mg/kg	milligram per kilogram
mg/kg/day	milligram per kilogram per day
mg/L	milligram per liter
mm Hg	millimeter of mercury
MRID	Master Record Identification Number
MTI	2H-Cyclopent(d)isothiazol-3(4H)-one, 5,6-dihydro-2-methyl-
N/A	not applicable
nm	nanometers
OCSPP	Office of Chemical Safety and Pollution Prevention
OPP	Office of Pesticide Programs
%	percent
PC Code	Pesticide Chemical Code
pH	power of hydrogen or power of the concentration of the hydrogen ion
ppm	parts per million
PWP	Preliminary Work Plan
RED	Reregistration Eligibility Decision
SAR	structure activity relationship
TGAI	technical grade active ingredient
TMDL	total maximum daily loads
U.S. EPA	United States Environmental Protection Agency
UV/VIS	ultraviolet/visible light absorption
WWTPs	wastewater treatment plants

Table of Contents

List of Tables

1 Introduction

This document is the Environmental Protection Agency's (EPA or "the Agency") Interim Registration Review Decision for 2H-Cyclopent(d)isothiazol-3(4H)-one, 5,6-dihydro-2-methyl-, herein referred to as MTI, and is being issued pursuant to 40 CFR Sections 155.56 and 155.58. A registration review decision is the Agency's determination whether a pesticide meets, or does not meet, the standard for registration in the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). The Agency may issue, when it determines it to be appropriate, an interim registration review decision before completing a registration review. Among other things, the interim registration review decision may require new risk mitigation measures, impose interim risk mitigation measures, identify data or information required to complete the review, and include schedules for submitting the required data, conducting the new risk assessment and completing the registration review. For further information on MTI, additional documents can be found in EPA's public docket (EPA-HQ-OPP-2015-0266) at www.regulations.gov.

The Proposed Interim Registration Review Decision for MTI was issued for public comment on October 10, 2015 and closed on December 7, 2015. During the 60-day public comment period, no comments were received concerning the registration review of MTI.

1.1 Statutory and Regulatory Authority

FIFRA, as amended by the Food Quality Protection Act (FQPA) of 1996, mandated the continuous review of existing pesticides. All pesticides distributed or sold in the United States generally must be registered by the EPA based on scientific data showing that they will not cause unreasonable risks to human health or the environment when used as directed on product labeling. The registration review program is intended to make sure that, as the ability to assess risk evolves and as policies and practices change, all registered pesticides continue to meet the statutory standard of no unreasonable adverse effects to human health or the environment. Changes in science, public policy, and pesticide use practices will occur over time. Through the registration review program, the Agency periodically reevaluates pesticides to make sure that as change occurs, products in the marketplace can continue to be used safely. Information on this program is provided at http://www2.epa.gov/pesticide-reevaluation. In 2006, the Agency implemented the registration review program pursuant to FIFRA section 3(g) and will review each registered pesticide every 15 years to determine whether it continues to meet the FIFRA standard for registration.

1.2 Case Overview

The docket for MTI (case 5018) has been established at http://www.regulations.gov in docket number EPA-HQ-OPP-2015-0266. Documents associated with this registration review can be viewed in this docket. Tables 1-2 below summarize the issues relevant to this registration review case and the registration review schedule.

Table 1 – Summary of Risk Assessments and Data Needs for Registration Review: MTI

Risk Assessment	Assessment Necessary to Support Registration Review	Date of Most Recent Assessment	Type of Assessment Required (New/Updated)	Data Needed
Dietary (food/ drinking water)	No	N/A	None	None
Occupational & Residential Handler / Post Application	No	N/A	None	None
Aggregate	No	N/A	None	None
Cumulative	No	N/A	None	None
Tolerance Review	No	N/A	None	None
Ecological	No	N/A	None	None

N/A = Not applicable

Table 2 – Registration Review Schedule

Activity	Target Date	Completion Date
Combined Preliminary Work Plan and Interim Registration Review Decision and Implementation		
Open 60-Day Public Comment Period for Combined Preliminary Work Plan and Proposed Interim Decision	2015-09	2015-10-07
Close Public Comment Period	2015-11	2015-12-07
Issue Interim Decision and Begin Post-Decision Follow up*	2016-03	2016-03
Total (years)	1	

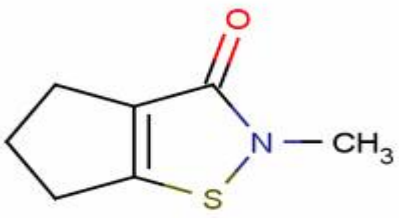
* A final decision on the MTI registration review case, in accordance with 40 CFR Section 155.58, will occur after an EDSP FFDCA section 408(p) determination has been made.

1.3 Chemical Identification and Properties

Table 3 presents the active ingredient to be assessed in case 5018: MTI (PC Code 107107).

Table 3 – Chemical Identification of MTI

Chemical Name	MTI
Chemical Classification	Isothiazolone
PC Code	107107
CAS Number	82633-79-2
Molecular Formula	C_7H_9NOS
Molecular Weight (grams/mole)	155.22

Molecular Structure	

The MTI physical chemistry information is summarized in Table 4.

Table 4 – Physical-Chemical Properties for MTI

Guideline Numbers	MTI	Source
830.6303 Physical State	Straw-colored liquid	43678303
830.7000 pH at 25 °C	4.8	43678303
830.7050 UV/Visible Absorption	Limited light absorption between 290-800 nm	43138707
830.7300 Density (g/cm^3)	1.016	43678303
830.7370 Dissociation Constant pKa and pKb	5.7 (pKa) 8.3 (pKb)	43138706
830.7550 Log *Kow*	0.64	EPIWEB 4.11
830.7840 Solubility in water (mg/L) at 25 °C	20,400	EPIWEB 4.11
830.7950 Vapor pressure (mm Hg) at 25 °C	6.62×10^{-4}	EPIWEB 4.11
Henry's law (atm m^3 mol^{-1})	6.62×10^{-9}	Calculated

1.4 Use/Usage Description

1.4.1 Registrations

There is one EPA registered product containing MTI as an active ingredient.[1] The product contains five percent MTI and is a liquid formulation.

1.4.2 Summary of Registered Uses

Table 5 presents a summary of the registered use of MTI that will be assessed in this registration review.

[1] Promexal X-50 Preservative, EPA Reg. No. 59441-5

Table 5 – Summary of MTI Registered Uses

Use - Material Preservation	Application Method	Application Rate
A preservative for use in the manufacture of aqueous compositions used in the manufacture of imaging products.	Open Pour	100 ppm

1.4.3 Usage Information

The amount of MTI used per year is very low. The exact amount is provided in a confidential report (MRID 49677601).

1.5 Regulatory History

The first product containing MTI was registered on July 23, 1996 for material preservation in the manufacture of aqueous compositions used in the manufacture of imaging products. A Reregistration Eligibility Decision (RED) was not completed for MTI because it was registered after November 1, 1984.

1.5.1 Recent/Pending Regulatory Actions

There are no recent or pending regulatory actions for MTI.

1.5.1 Tolerance Information

EPA has not established tolerances or tolerance exemptions for residues of MTI in or on raw agricultural commodities or processed food and feed products under the Federal Food, Drug, and Cosmetic Act (FFDCA) Section 408. MTI has not been cleared as a food additive by the U.S. Food and Drug Administration (US FDA) under FFDCA Section 409.

1.6 Incidents

1.6.1 Human Health

There are no human health incidents in the OPP Incident Data System for the time period from 8/06/2001, when the product was transferred from Zeneca to Kodak, to the present (search conducted on July 27, 2015). There is one incident listed for when the product was registered to Zeneca. This incident was included in the first quarter aggregate report for 2000 and was rated as grade D (minor). No other information was provided in the aggregate report.

1.6.2 Ecological

There are no ecological incidents in the OPP Incident Data System as of July 21, 2015.

2 Anticipated Data Needs

The Agency does not require any additional data to support this registration review.

3 Human Health Risk Assessment

The Agency does not need to conduct a human health risk assessment for MTI.

3.1 Existing Toxicological Endpoints

Based on the required personal protective equipment (PPE) and engineering controls, there are no toxicological endpoints of concern and no points of departure are set. The requirement for a subchronic inhalation study, 90-day dermal toxicity study, rabbit developmental study, reproduction and fertility study, dominant lethal mutation assay and immunotoxicty assay were reviewed by the Hazard and Science Policy Council (HASPOC) on July 23, 2015 and waived (TXR# 0057278) based on limited usage, PPE, and engineering controls used.

3.2 Dietary Exposure

The Agency does not need to conduct a dietary exposure assessment to support this registration review.

3.2.1 Food and Drinking Water

A dietary (food and drinking water) exposure assessment is not required for MTI at this time. MTI is used as a preservative for the manufacture of aqueous compositions used in the manufacture of imaging products and is not expected to result in dietary exposures. Therefore, a dietary exposure assessment is not required.

3.3 Occupational and Residential Exposures

3.3.1 Occupational Exposures

Occupational handler exposures could occur during the open pouring of the MTI product from the original containers into smaller containers and from the smaller containers into batches of film emulsion. The label indicates, however, that all handling of the MTI product must be done under local exhaust ventilation and thus inhalation exposures are anticipated to be negligible. In addition, dermal exposures are also expected to be negligible given the small amounts handled and the use of PPE including rubber gloves and goggles or a faceshield.

Occupational post application exposures to MTI residues on the film during color film developing are also not anticipated because color film developing is an automated process that involves immersion in several chemical solutions followed by a water wash and drying.

3.3.2 Residential Exposures

Residential exposures are not anticipated because MTI is used only in an industrial process to manufacture imaging products (i.e. film) which is then developed using an automated process.

3.4 Aggregate and Cumulative Exposure

3.4.1 Aggregate Exposures

Aggregate exposures normally include dietary, drinking water and residential exposures. Since these exposures are not anticipated for MTI, a risk assessment of aggregate MTI exposures is not needed.

3.4.2 Cumulative Exposures

Dietary, drinking water and residential exposures are not anticipated for MTI, thus a risk assessment of cumulative exposures to MTI and toxicologically similar chemicals is not needed.

4 Environmental Risk Assessment

The Agency does not need to require data or to conduct an environmental risk assessment for MTI. The Agency has not previously conducted a risk assessment that supports a complete endangered species determination for MTI; further, no assessment is needed at this time due to lack of potential exposure. Waste materials that are generated following the use of MTI as a preservative undergo incineration as part of the recovery of silver from the solution. Consequently, disposal of waste materials is expected to be negligible and the potential for ecological organisms to be exposed to MTI is expected to be negligible.

4.1 Threatened and Endangered Species

There is no reasonable expectation for the registered use of MTI to cause direct or indirect adverse effects to threatened and endangered species. Usage is low and waste materials are incinerated. No environmental exposure is expected. No adverse modification of any designated critical habitat for such species is expected from the use of MTI. EPA has made a "no effect" determination for MTI under the Endangered Species Act (ESA) for all listed species and designated critical habitat for such species and has therefore concluded that consultation with the Fish and Wildlife Service and the National Marine Fisheries Service under ESA section 7(a)(2) is not required.

4.2 Environmental Fate Assessment

MTI is an isothiazolone microbiocide which is stable to hydrolysis at pH 5, 7, and 9 and 25 ^{o}C (MRID 43138732). Although no guideline study on photodegradation in water was performed, MTI is expected to be relatively stable to photodegradation in water based on results from a UV-visible absorption study. Only 7 % of MTI degraded within 24 hours of the onset of a UV-visible absorption study (MRID 43138707, Guideline 830.6313), which indicated that photodegradation is expected to be negligible. No other fate data have been submitted, but no data are necessary based on use pattern and industry practices.

4.2.1 Water Quality

MTI is not identified as a cause of impairment for any water bodies listed as impaired under section 303(d) of the Clean Water Act.[2] In addition, no Total Maximum Daily Loads (TMDL) have been developed for MTI.[3] More information on impaired water bodies and TMDLs can be found at EPA's website.[4]

4.3 Ecological Effects Assessment

4.3.1 Ecotoxicology Endpoints

Acute and chronic toxicity data from registrant-submitted studies (850 OCSPP Harmonized Test Guidelines) are used to evaluate the potential effects to aquatic and terrestrial nontarget organisms. The available data indicate that technical-grade MTI is moderately to highly toxic to freshwater organisms and moderately to practically nontoxic to birds (Table 10). No additional ecotoxicity data are needed.

Table 10. Available Ecological Effects Endpoints

Receptor Group	Test Material	Scenario	Toxicity Endpoint (µg/L)	Reference
Freshwater fish	TGAI	Acute	LC50 = 0.89 mg ai/L	43138712
Freshwater invertebrates	TGAI	Acute	EC50 = 1.3 mg ai/L	43138713
Birds	TGAI	Acute	LD50 = 152 mg/kg-bw	43138708
		Dietary	LC50 >5620 ppm	43138709, 43138710

4.4 Exposure Analysis Plan

4.4.1 Aquatic and Terrestrial Wildlife Exposure Estimates

Aquatic and terrestrial exposures are not anticipated from the use of MTI as a preservative. Consequently, no aquatic and terrestrial assessments of exposure are being performed.

4.4.2 Screening Level Down-the-Drain Analysis

No Down-the-Drain (DtD) releases to wastewater treatment plants (WWTP) from the use of MTI as a preservative. Consequently, no DtD assessment is being conducted for MTI.

[2] http://iaspub.epa.gov/tmdl_waters10/attains_nation_cy.cause_detail_303d?p_cause_group_id=885
[3] http://iaspub.epa.gov/tmdl_waters10/attains_nation.tmdl_pollutant_detail?p_pollutant_group_id=885&p_pollutant_group_name=PESTICIDES
[4] http://www.epa.gov/tmdl/

5 Endocrine Disruptor Screening Program (EDSP)

As required by FIFRA and FFDCA, EPA reviews numerous studies to assess potential adverse outcomes from exposure to chemicals. Collectively, these studies include acute, subchronic and chronic toxicity, including assessments of carcinogenicity, neurotoxicity, developmental, reproductive, and general or systemic toxicity. These studies include endpoints which may be susceptible to endocrine influence, including effects on endocrine target organ histopathology, organ weights, estrus cyclicity, sexual maturation, fertility, pregnancy rates, reproductive loss, and sex ratios in offspring. For ecological hazard assessments, EPA evaluates acute tests and chronic studies that assess growth, developmental and reproductive effects in different taxonomic groups. As part of its most recent registration decision, for MTI, EPA reviewed these data and selected the most sensitive endpoints for relevant risk assessment scenarios from the existing hazard database. However, as required by FFDCA section 408(p), MTI is subject to the endocrine screening part of the Endocrine Disruptor Screening Program (EDSP).

EPA has developed the EDSP to determine whether certain substances (including pesticide active and other ingredients) may have an effect in humans or wildlife similar to an effect produced by a "naturally occurring estrogen, or other such endocrine effects as the Administrator may designate." The EDSP employs a two-tiered approach to making the statutorily required determinations. Tier 1 consists of a battery of 11 screening assays to identify the potential of a chemical substance to interact with the estrogen, androgen, or thyroid (E, A, or T) hormonal systems. Chemicals that go through Tier 1 screening and are found to have the potential to interact with E, A, or T hormonal systems will proceed to the next stage of the EDSP where EPA will determine which, if any, of the Tier 2 tests are necessary based on the available data. Tier 2 testing is designed to identify any adverse endocrine-related effects caused by the substance, and establish a dose-response relationship between the dose and the E, A, or T effect.

Under FFDCA section 408(p), the Agency must screen all pesticide chemicals. Between October 2009 and February 2010, EPA issued test orders/data call-ins for the first group of 67 chemicals, which contains 58 pesticide active ingredients and 9 inert ingredients. A second list of chemicals identified for EDSP screening was published on June 14, 2013[5] and includes some pesticides scheduled for registration review and chemicals found in water. Neither of these lists should be construed as a list of known or likely endocrine disruptors.

For further information on the status of the EDSP, the policies and procedures, the lists of chemicals, future lists, the test guidelines and the Tier 1 screening battery, please visit our website.[6]

6 Interim Registration Review Decision

In accordance with 40 CFR Sections 155.56 and 155.58, the Agency is issuing this Interim Registration Review Decision document. Except for the EDSP component of the MTI

[5] See http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPPT-2009-0477-0074 for the final second list of chemicals.
[6] http://www2.epa.gov/endocrine-disruption

registration review case, the Agency has made the decision that no additional data are required and that there are no human health and ecological risk concerns based on the assumption of no exposure to humans and non-target organisms. The Agency has made an ESA "no effect" finding based on lack of potential exposure for endangered species (see Section 4.1).

7 Next Steps

A Federal Register Notice will announce the availability of this Interim Registration Review Decision for MTI. The final decision on the registration review for MTI (case 5018) will occur after an EDSP FFDCA section 408(p) determination is made.

8 References

43138718 McCall, J. (1990) 2-Methyl-4,5-Trimethylene-4-Isothiazolin-3-One: Acute Oral Toxicity to the Rat: Lab Project Number: CTL/P/2791. Unpublished study prepared by ICI Central Toxicology Lab. 49 p.

43138719 McCall, J.; Leah, A. (1990) 2-Methyl-4,5-Trimethylene-4-Isothiazolin-3-One: Acute Dermal Toxicity to the Rat: Lab Project Number: CTL/P/2946. Unpublished study prepared by ICI Central Toxicology Lab. 37 p.

43138720 Barlow, A.; Leah, A. (1990) 2-Methyl-4,5-Trimethylene-4-Isothiazolin-3-One (MTI): Eye Irritation to the Rabbit: Lab Project Number: CTL/P/2811. Unpublished study prepared by ICI Central Toxicology Lab. 21 p.

43138721 Barlow, A.; Leah, A. (1990) 2-Methyl-4,5-Trimethylene-4-Isothiazolin-3-One (MTI): Skin Irritation to the Rabbit: Lab Project Number: CTL/P/2810. Unpublished study prepared by ICI Central Toxicology Lab. 18 p.

43138722 Lees, D. (1990) 2-Methyl-4,5-Trimethylene-4-Isothiazolin-3-One (MTI): Skin Sensitization to the Guinea Pig: Lab Project Number: CTL/P/2960. Unpublished study prepared by ICI Central Toxicology Lab. 31 p.

43138724 Rattray, N. (1993) 2-Methyl-4,5-trimethylene-4-isothiazolin-3-one (MTI): 90 Day Feeding Study in Rats: Lab Project Nos. CTL/P/3858: PR0903. Unpublished study prepared by Zeneca Central Toxicology Lab. 537 p.

43138726 Rattray, N. (1993) 2-Methyl-4,5-Trimethylene-4-Isothiazolin-3-One (MTI): 90 Day Toxicity Study in Dogs: Lab Project Number: CTL/P/3972: PD0931. Unpublished study prepared by Zeneca Central Toxicology Lab. 342 p.

43138727 Pinto, P. (1993) 2-Methyl-4,5-trimethylene-4-isothiazolin-3-one (MTI): Developmental Toxicity Study in the Rat: Lab Project Number: CTL/P/3921: RR0603. Unpublished study prepared by Zeneca Central Toxicology Lab. 375 p.

43138728 Calander, R.; Priestley K. (1990) 2-Methyl-4,5-Trimethylene-4-Isothiazolin-3-One: An Evaluation of Mutagenic Potential Using S. typhimurium and E. coli: Lab Project Number: CTL/P/2844. Unpublished study prepared by ICI Central Toxicology Lab. 40 p.

43138729 Jones, K.; Mackay, J. (1990) 2-Methyl-4,5-trimethylene-4- isothiazolin-3-one (MTI): An Evaluation in the Mouse Micronucleus Test: Lab Project Number: CTL/P/3132. Unpublished study prepared by ICI Central Toxicology Lab. 40 p.

43138730 Jones, K.; Mackay, J. (1990) 2-Methyl-4,5-Trimethylene-4-Isothiazolin-3-One (MTI): An Evaluation in the In Vitro Cytogenetic Assay in Human Lymphocytes: Lab Project Number: CTL/P/3035. Unpublished study prepared by ICI Central Toxicology Lab. 34 p.

43138731 Kennelly, J. (1990) 2-Methyl-4,5-Trimethylene-4-Isothiazilin-3-One (MTI): Assessment for the Induction of Unscheduled DNA Synthesis in Rat Hepatocytes In Vivo: Lab Project Number: CTL/P/3134. Unpublished study prepared by ICI Central Toxicology Lab. 43 p

49677601 Occupational Exposure Assessment of the Use of Promexal X50 as a Biocide in Kodak Color Negative Film Products, John H. Heyer, Eastman Kodak Company, June 10, 2015.

Appendix A Toxicology Profile

Acute Toxicity for Product Labeling

Table 6 – Acute Toxicity Studies for MTI

Guideline No./ Study Type	MRID No.	Results	Toxicity Category
870.1100/ Acute oral toxicity	43138718	LD50 = 224 (M) and 168 (F) mg/kg for males and females	II
870.1200/ Acute dermal toxicity	43138719	Animals sacrificed due to severity of skin irritation	I
870.1300/ Acute inhalation toxicity	N/A	No data performed	I
870.2400/ Acute eye irritation	43138720	Animals sacrificed due to severity of eye irritation	I
870.2500/ Acute dermal irritation	43138721	No erythema or edema seen at 72 hours	IV
870.2600/ Skin sensitization	43138722	Sensitizer	Not applicable

N/A=Not available

Subchronic Toxicity

There are two subchronic toxicity studies, 1 in rat and 1 in dogs, both are considered acceptable.

90-day feeding study rat – MRID 43138724

MTI was administered to AlpK:APfsd (Wistar derived) rats of both sexes for a period of 90 days in the diet at dose levels of 0, 50, 250, or 1000 ppm corresponding to mean MTI intakes of 0, 4.1, 20.7 or 83 mg/kg/day (M) or 0, 4.6, 23.2, or 93 mg/kg/day (F). Statistically significantly decreased body weights were observed at 1000 ppm in males throughout the treatment period (e.g. to 84.8% of controls at 14 weeks). Mean body weight gains at 250 and 1000 ppm in males were reduced to 95.2 and 79.3% of controls for weeks 1-14. Mean food efficiency values for high dose males were statistically significantly decreased for weeks 1-13 (to 89.7% of controls). Statistically significantly decreased body weights were observed at 250 and 1000 ppm in females throughout the treatment period (e.g. to 94.5 and 87.8% of controls at 14 weeks for 250 and 1000 ppm, respectively). Likewise, mean body weight gains decreased in dose related fashion to 89.1% and 68.7% of controls at 250 and 1000 ppm, respectively, for weeks 1-14. Mean food efficiency values for weeks 1-4 decreased with increasing dose, reaching statistical significance at 250 and 1000 ppm, at 91.3 and 75.6% of controls, respectively. There were no apparent toxicologically significant findings in clinical chemistry, hematology, or macroscopic and microscopic pathology.

LOEL: 250 ppm, based on dose related and statistically significant decreases in food efficiency for females coupled to dose related decreases in body weight gain.

NOEL: 50 ppm.

90-day feeding – dogs – MRID 43138726

In a 13-week subchronic feeding study (MRID# 431387-26) MTI was administered to beagle dogs of both sexes for a period of 90 days in the diet at dose levels of 0, 100, 300, or 1000 ppm, corresponding to mean MTI intake of 0, 3.1, 9.3 or 31.5 mg/kg/day in males and 0, 3.2, 10.1, or 33.4 mg/kg/day in females. There were no effects on body weight or body weight gain for either sex. This is in contrast with effects observed in the pilot study (MRID 43138725): body weight gain in females at 928 and 464 ppm in the pilot study were 36.4% and 54.5% of controls, respectively. Changes in hematology and clinical chemistry parameters did not follow a clear dose related trend and appeared to be incidental to the treatment. No clear-cut, dose-related effects were observed upon macroscopic examination of the dogs. Although clear macroscopic signs of irritation of the tongue were seen in 1392 and 928 ppm females in the pilot study, no signs of tongue irritation were seen in this study. There were no apparent treatment-related histological findings. This study does not define a LOEL due to the absence of clear-cut, dose-related effects. The NOEL is 1000 ppm, the highest dose tested.

Developmental Toxicity

There is one developmental toxicity study in rats. The developmental toxicity study in rabbits was waived by HASPOC (TXR# 0057278).

Developmental toxicity study – rat – MRID 431387-27

A developmental toxicity study (MRID# 431387-27) was conducted with Wistar-derived rats (Alpk:APfsD)(SPF) to assess the developmental toxicity potential of MTI in mated female rats (groups of 24/dose) gavaged during gestation days (gds) 7-16 (inclusive) at dose levels of 0, 5, 15 or 40 mg/kg/day. Maternal toxicity was established for the highest dose tested (HDT) by: 1) the observation of 2 deaths on gds 12 and 13 (1 in extremis, 1 found dead), 2) Clinical signs of toxicity in 4 dams (including the two which died) related to the respiratory system (abnormal noise, labored breathing, gasping, reduced breathing rate), 3) statistically significant depressions in mean body weight of 4-6.5% (gds 8-16 inclusive) which remained decreased following cessation of dosing, 4) a 62 % decrement in mean body weight gain during the dosing period, and 5) statistically significant depressions in mean food consumption on gds 7-10, 10-13 and 13-16. There were no apparent dose-related effects upon mean implantations/dam, live fetuses/dam, resorptions/dam (early, late), postimplantation losses, the sex ratios, mean fetal weights or external/ visceral/skeletal anomalies. The Maternal toxicity NOEL is equal to 15 mg/kg/day while the LOEL is equal to 40 mg/kg/day. (HDT). This is based upon increased mortality and clinical signs (respiratory system), depressed mean body weight and weight gain and decreased food consumption. The Developmental NOEL is equal to 40 mg/kg/day (HDT) while the LOEL could not be determined.

Reproductive Toxicity

This requirement was waived by HASPOC (TXR# 0057278).

Chronic Toxicity

This study is not required for this use pattern.

Carcinogenicity

This study is not required for this use pattern.

Mutagenicity

The database for mutagenicity is considered adequate. Four mutagenicity studies were conducted and an additional dominant lethal assay was waived.

In an initial microbial/mammalian microsome plate incorporation assay (MRID No. 43138731) *Salmonella typhimurium* strains TA 1535, TA 1537, TA 1538, TA98, and TA 100, and *Escherichia coli* strain WP2uvrA pKM101 were exposed to 0, 0.32, 1.6, 8.0, 40, 100, or 200 µg/plate of MTI in the presence or absence of SS activation derived from Arochlor 1 254 induced rat liver. For the repeat trial, nonactivated levels of 0, 0.16, 0.8, 4.0, 20, 50, or 100 µg/plate were assayed with the Salmonella strains and 0, 0.064, 0.32, 1.6, 8.0, 20, or 50 pg/plate were assayed with E. coli Doses evaluated under SS-activated conditions were similar to those used in the initial trial. Dimethyl sulfoxide was used as the solvent. Although slight but significant and dose-related increases in revertant colonies of strain TA 1 535 were seen in the SS-activated phase of the initial assay, the effect was not reproducible and, therefore, did not provide sufficient evidence of a mutagenic response. MTI was cytotoxic (~ 100 µg/plate -SS; ~200 pg/plate + S9) in all strains but failed to induce a mutagenic effect. All strains responded to the mutagenic action of the appropriate positive control.

In an *in vivo*/*in vitro* unscheduled DNA synthesis (UDS) study (MRID No. 43138731), groups of five male rats were administered a single oral gavage dose of 0, 76, 117, or 180 mg/kg MTI prepared in deionized water. The high dose was estimated to be 80% of the male rat oral LD_{50}. Animals were sacrificed at 4 and 1 2 hours post-treatment and recovered hepatocytes were scored for UDS. Two independent trials were performed. Clinical signs of toxicity noted immediately after dosing included salivation, difficulty in breathing, and staining and fluid around the nose. Treatment with MTI produced no evidence of cytotoxicity for the target cells. There was also no evidence of a genotoxic response at any dose or sacrifice time.

In *an in vitro* cytogenetic assay (MRID No. 43138730), cultured human lymphocytes, obtained from one male and one female donor were exposed to MTI doses of 0, 2, 10, or 20 pg/ml -S9 (male donor); 0, 1, 5, or 10 µg/ml -S9 (female donor) or 0, 2, 10, or 20 µg/ml + S9 (both donors) (MRID No. 431387-30, HED Doc. No. 011613) . The test material was delivered to the test system in dimethyl sulfoxide, and the S9 was derived from Aroclor 1254 induced rat liver. Significant ($p < 0.01$) clastogenic effects were seen at 10 and 20 pg/ml without S9 activation and at 20 µg/ml with S9 activation in lymphocytes derived from the male donor and at 10 and 20 pg/ml + S9 in cells derived from the female donor. Higher levels with or without S9 activation were severely cytotoxic. MTI is considered positive for the induction of structural chromosomal aberrations in cultured human lymphocytes. Cultured human lymphocytes tested positive for

structural chromosomal aberrations in the presence and absence of metabolic activation at concentrations as low as 10 microgram/ml. The response was much more pronounced in the presence of metabolic activation.

In the mouse micronucleus assay (MRID No. 43138729) MTI tested weakly positive at a dose level up to 136 mg/kg. In this assay, groups of five male and five female C57BL/6 mice received single oral gavage administrations of 0, 85 or 136 mg/kg (males) or 0, 103 or 164 mg/kg (females) MTI delivered in physiological saline. At 24, 48 or 72 hours postexposure, high dose animals were sacrificed and bone marrow cells were examined for micronucleated polychromatic erythrocytes (MPEs). Bone marrow cells were harvested from animals in the low-dose groups only at 24 hours after treatment. Unscheduled deaths occurred in five high-dose males and four high-dose females; dead animals were replaced with mice from a secondary group. A significant ($p < 0.05$) decrease in the ratio of normochromatic to polychromatic erythrocytes (PCEs), indicating cytotoxic effects on the target organ, was seen in the high-dose groups (both sexes) at the 24-hour sacrifice. A significant ($p<0.01$) increase in mean MPEs for males administered 136 mg/kg at 24 hours prompted the study investigators to evaluate an additional 2000 PCEs/male from the 24- and 48-hour sacrifice groups for the high dose and from the 24-hour sacrifice group for the low dose. Based on this expanded analysis, the data showed nonsignificant increases in MPEs for high-dose males at both sacrifice times. A similar response was present in high-dose females at 48 hours. The study authors attributed the increase in males (48 hr.) to a single animal having high MPE counts (14/1000--initial count; 13/1000 and 12/1000 –subsequent extended counts). However, increases in the number of micronuclei were seen in individual animals as follows:

2 high-dose males (24 hrs)--4.3 or 4.0 MPEs/1000 PCEs vs. 2.1 MPEs/1000 PCEs (controls)
2 high-dose males (48 hrs)--3 .3 or 13.0 MPEs/1000 PC Es vs. 1. 7 MPEs/1000 PCEs (control)
1 low-dose male (24 hrs)--4. 7 MPEs/PCEs vs. 2. 1 MPEs/1000 PC Es (controls)
1 high-dose female (48 hrs)--10 MPEs/1000 PCEs vs. 1 .8 MPEs/1000 PCEs.

Although the statistical significance of the results was lost owing to the wide variability in the data, the increased sample size (3000 PCEs/males at all doses and sacrifice times) increases our confidence in the test system's ability to predict a doubling over background as a true biological effect. It is also of note that MTI induced an unambiguous clastogenic response in cultured human lymphocytes (see MRID No.431387-30). It is concluded, therefore, that the results in this mouse micronucleus assay are consistent with a weak clastogenic and/or aneugenic response.

Other Toxicological Effects

Immunotoxicity study was waived by HASPOC (TXR# 0057278). Acute and subchronic neurotoxicty studies are not required for this use pattern.

Appendix B Environmental Fate

Environmental Fate and Transport Properties of MTI

MTI is an isothiazolone microbiocide. The guideline environmental fate data that have been submitted for hydrolysis indicates stability at pH 5, 7, and 9 and 25 ^{o}C (MRID 43138732). Although no guideline study on photodegradation in water was performed, MTI is expected to be relatively stable to photodegradation in water based on results from a UV-visible absorption study. Only 7 % of MTI degraded within 24 hours of the onset of a UV-visible absorption study (MRID 43138707, Guideline 830.6313), which indicated that photodegradation is negligible.

No additional environmental fate data have been submitted for MTI, but no data are needed because of the lack of environmental exposure.

Environmental Fate References for Appendix B

MRID 43138707. Comber, M.; Roberts, G.; Woods, C. (1993) MTI: Photostability: Lab Project Number: BL4892/B. Unpublished study prepared by Brixham Environmental Labs., Zeneca Ltd. 16 p.

MRID 43138732. Brown, D.; Stanley, R. (1992) 2-Methyl-4,5-Trimethylene-4-Isothiazolin-3-One (MTI): Determination of Hydrolysis as a Function of pH: Lab Project Number: BL4450/B. Unpublished study prepared by Imperial Chemical Industries PLC, Brixham Lab. 11 p.